JC759 U.S. PTO
04/18/00

SB box

NONPROVISIONAL PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

OLIFF & BERRIDGE, PLC
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Attorney Docket No.: 105997

Date: April 18, 2000

BOX PATENT APPLICATION

NONPROVISIONAL APPLICATION TRANSMITTAL
RULE §1.53(b)

JC542 U.S. PTO
09/551645
04/18/00

Director of the U.S. Patent and Trademark Office
Washington, D.C. 20231

Sir:

Transmitted herewith for filing under 37 C.F.R. §1.53(b) is the nonprovisional patent application

For (Title): NEW EPITOPE TAG RECOGNIZED BY A MONOCLONAL ANTIBODY TO RICKETTSIA TYPHI

By (Inventors): Myong-Joon HAHN

☒ Formal drawings (Figs. 1-4; 2 sheets) are attached.
☒ A Declaration and Power of Attorney is filed herewith.
☐ An assignment of the invention to _____ is filed herewith.
☐ An Information Disclosure Statement is filed herewith.
☒ A statement to establish small entity status under 37 C.F.R. §§1.9 and 1.27 is filed herewith.
☒ A Preliminary Amendment is filed herewith.
☐ Please amend the specification by inserting before the first line the sentence --This nonprovisional application claims the benefit of U.S. Provisional Application No. _____, filed _____.--
☐ Priority of foreign application(s) No. _____ filed _____ in _____ is claimed (35 U.S.C. §119).
☐ A certified copy of the above corresponding foreign application(s) is filed herewith.
☒ The filing fee is calculated below:

CLAIMS IN THE APPLICATION AFTER ENTRY OF ANY PRELIMINARY AMENDMENT NOTED ABOVE

FOR:	NO. FILED	NO. EXTRA	SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY FEE
BASIC FEE				$ 345	OR		$ 690
TOTAL CLAIMS	5 - 20	= 0	x 9 =	$	OR	x 18	$
INDEP CLAIMS	2 - 3	= 0	x 39 =	$	OR	x 78	$
☐ MULTIPLE DEPENDENT CLAIMS PRESENTED			+130 =	$	OR	+260	$
			TOTAL	$345	OR	TOTAL	$

* If the difference is less than zero, enter "0".

☒ Check No. 107868 in the amount of $345 to cover the filing fee is attached. Except as otherwise noted herein, the Director is hereby authorized to charge any other fees that may be required to complete this filing, or to credit any overpayment, to Deposit Account No. 15-0461. Two duplicate copies of this sheet are attached.

☒ This application is entitled to small entity status. DO NOT charge large entity fees to our Deposit Account.

Respectfully submitted,

James A. Oliff
Registration No. 27,075

Melanie L. Mealy
Registration No. 40,085

JAO:MLM/jca

10/98

Applicant or Patentee: Myong-Joon HAHN
Serial or Patent No.: Attorney Docket No.: 105997
Filed or Issued: April 17, 2000
For:

STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27(b))-INDEPENDENT INVENTOR

As a below named inventor, I qualify as an independent inventor as defined in 37 CFR 1.9(c), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled:

New epitope tag recognized by a monoclonal antibody to Rickettsia typhi

described in:

☒ the specification filed herewith.
☐ application serial no. _____, filed _____.
☐ patent no. _____, issued _____.

I have not assigned, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

☐ no such person, concern, or organization.
☐ persons, concerns or organizations listed below:*

NOTE: Separate statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27).

FULL NAME Myong-Joon HAHN
ADDRESS Basic Medical Sciences, Sungkyunkwan University School of Medicine, Suwon, Kore
☒ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME _____
ADDRESS _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 CFR 1.28(b)).

Myong-Joon HAHN		
TYPED NAME OF INVENTOR	TYPED NAME OF INVENTOR	TYPED NAME OF INVENTOR
Myong-Joon Hahn		
Signature of Inventor	Signature of Inventor	Signature of Inventor
April 3, 2000		
Date	Date	Date

11/97

Inventor Information

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Country of Residence::	
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Family Name::	
Name Suffix::	
City of Residence::	
State or Prov. of Residence::	
Country of Residence::	
Inventor Four Given Name::	
Family Name::	
Name Suffix::	
City of Residence::	
State or Prov. of Residence::	
Country of Residence::	
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Application Information

Title Line One::	NEW EPITOPE TAG RECOGNIZED BY A
Title Line Two::	MONOCLONAL ANTIBODY TO RICKETTSIA
Title Line Three::	TYPHI
Title Line Four::	

Total Drawing Sheets:: 2
Docket Number:: 105997

Continuity Information

>This application is a::
Application One::
Filing Date::
Patent Number::
which is a::
>>Application Two::
Filing Date::
Patent Number::

Prior Foreign Applications

Foreign Application One::
Filing Date::
Country::
Priority Claimed::
Foreign Application Two::
Filing Date::
Country::
Priority Claimed::
Foreign Application Three::
Filing Date::
Country::
Priority Claimed::

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

BOX: SEQUENCE

Myong-Joon HAHN

Application No.: New U.S. Patent Application

Filed: April 18, 2000

Docket No.: 105997

For: NEW EPITOPE TAG RECOGNIZED BY A MONOCLONAL ANTIBODY TO *RICKETTSIA TYPHI*

PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Please delete the present Sequence Listing, pages 11-12 of the specification.

Please renumber pages 13 and 14 as pages 11 and 12, respectively.

At the end of the application, please insert the attached paper and computer readable Sequence Listing.

REMARKS

The attached paper copy and computer readable copy of the Sequence Listing are submitted in compliance with 37 C.F.R. §§1.821-1.825. The contents of the paper copy and the computer readable copy of the Sequence Listing are the same. Support for the information provided in the Sequence Listing can be found in the original Sequence Listing. No new matter is added.

Early and favorable consideration on the merits is respectfully requested.

Respectfully submitted,

James A. Oliff
Registration No. 27,075

Melanie L. Mealy
Registration No. 40,085

JAO:MLM/jca

Attachments:
Sequence Listing (paper and computer readable copy)

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TITLE OF THE INVENTION

NEW EPITOPE TAG RECOGNIZED BY A MONOCLONAL ANTIBODY TO *RICKETTSIA TYPHI*

BACKGROUND OF THE INVENTION

This invention relates to an epitope tag recognized by monoclonal antibody to the crystalline surface layer protein (SLP) to *Rickettsia typhi* and a method for detecting the tagged protein using immunoblotting, immunocytochemistry, and immunoprecipitation.

Epitope tagging and the antibody to the epitope have been widely used in cellular and molecular biology research. When no antibody to a particular protein is available, construction of a fusion gene containing a particular epitope (Tag) and subsequent detection of its product by the anti-Tag antibody are valuable alternatives for the characterization of that protein.

For example, epitope tagging has been applied to elucidate intracellular location, post-translational modification, affinity purification, and interactions with other proteins of the tagged protein. Further, the immunogenic and antigenic determinants of a synthetic peptide and the corresponding antigenic determinants in the parent protein have been elucidated [Evan et al., *Mol. Cell. Biol.*, **12**, pp3610-3616 (1985) ; Wilson et al., *Cell*, **37**, pp767-778 (1984)].

Furthermore, for the purpose of simultaneous expression of several ectopic genes and distinguishing the gene products from endogenous proteins, several different Tags along with sensitive and specific antibodies to such

Tags are required.

Previously, the inventor made mouse monoclonal antibodies to the crystalline surface layer protein (SLP) of *Rickettsia typhi* and cloned the gene (*slpT*) encoding this protein [Hahn et al., *Gene,* **133,** pp129-133 (1993)]. In this study, the inventor determined the epitope recognized by one of our monoclonal antibodies (SRT10, IgG2a) to ten amino acid residues of SLP. By tagging this epitope to a putative chloride channel protein, NCC27/CLIC1, which is not well characterized [Tulk et al., *Am. J. Physiol.*, **274,** pp1140-9 (1998) ; Valenzuela et al., *J. Biol. Chem.*, **272,** pp12575-82 (1997)], the inventor examined the usefulness of this epitope tag and SRT10 as tools for the molecular and cellular biology research.

SUMMARY OF THE INVENTION

The object of the invention provides an epitope recognized by a mouse monoclonal antibody (MAb) to the crystalline surface layer protein (SLP) of *Rickettsia typhi,* SRT10, which is mapped to ten amino acid residues (SRTag, Thr Phe Ile Gly Ala Ile Ala Thr Asp Thr [SEQ ID NO : 1]).

Another object of the invention provides an oligonucleotide sequence [SEQ ID NO : 2] covering the epitope recognized by SRT10, which is inserted to a mammalian expression vector together with multiple cloning sites.

Further object of the invention provides a monoclonal or polyclonal antibody to the SRTag as an epitope for any tagged protein.

When the SRTag is fused in frame to the coding region of any protein gene and expressed in bacteria or mammalian cells, the MAb SRT10 can

detect the tagged protein by immunoblotting, immunocytochemistry, and immunoprecipitation.

We suggest that this specific recognition of the SRTag by SRT10 is generally applicable to the research of cellular and molecular biology requiring the expression and detection of fusion proteins.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG 1. Construction of SRT-NCC27/CLIC1 plasmid. Oligonucleotides containing the coding region of SRTag and multiple cloning sites were inserted to pCMV6. cDNA of NCC27/CLIC1 was subcloned to the *Bam*HI/*Sal*I sites of the prepared expression plasmid.

FIG 2. SRT10 recognizes the tagged NCC27/CLIC1 by immunoblotting. Untransfected cell lysate was probed with 1 g/ml of SRT10 (lane 1). SRT-NCC27/CLIC1 transfected cell lysates were probed with 0.1 (lane 2), 1 (lane 3), 10 (lane 4), 100 (lane 5), or 1000 ng/ml (lane 6) of SRT10. Molecular size standards are indicated on the left in kilodoltons (kDa).

FIG 3. SRT10 precipitates the tagged NCC27/CLIC1 from mammalian cell lysates. In lane 1, 75 g of total cell lysate (50% of input) was loaded. 150 g of cell lysates were precipitated with control antibody (lane 2) or SRT10 (lane 3-7). Precipitated tagged protein and antibody complexes were washed with washing buffer containing 0.25 M (lane 2 and 3), 0.5 M (lane 4), 0.75 M (lane 5), 1 M (lane 6), or 1.5 M (lane 7) NaCl. Molecular size standards are indicated on the left in kilodoltons (kDa).

FIG 4. SRT10 recognizes intracellular SRT-NCC27/CLIC1. HeLa (A

and B) and C2C12 cells (C and D) were transfected with the expression plasmid of SRT-NCC27/CLIC1, stained with SRT10, and examined by confocal microscopy.

DETAILED DESCRIPTION OF THE INVENTION

MATERIALS AND METHODS

Epitope mapping and expression plasmid construct

A series of deletion constructs of the *slpT* gene of *R. typhi* were prepared in pGEX™-4T-1 (Pharmacia, Uppsala, Sweden). By examining the reactivity of the SRT10 to bacterial lysates transformed with the expression constructs, we determined the epitope recognized by this MAb. On determining the epitope, its DNA sequence was inserted to the *Sal*I/*Eco*RI sites of pCMV6 together with several cloning sites with oligonucleotides (FIG 1). To this modified pCMV6, PCR-amplified cDNA of NCC27/CLIC1 (3, 4) was inserted. Oligonucleotides used for PCR were as follows : 5′ GACGGATCCATGGCTGAAGAACAAC [SEQ ID NO : 3] ; and 5′ TCCCTCGAGGGGCTTATTTGAGGGC [SEQ ID NO : 4]. Underlining indicates the restriction sites (*Bam*HI and *Xho*I, respectively). The resulting PCR product was cloned into the *Bam*H1/*Sal*1 sites of the pGEX-4T-1 and the tag-inserted pCMV6, for bacterial and mammalian cell expressions, respectively. Authenticity of the constructs was confirmed by automatic nucleotide sequencing.

Preparation of rabbit polysera to the NCC27/CLIC1

Recombinant GST-NCC27/CLIC1 fusion protein was prepared from *E.*

coli. Purified GST-NCC27/CLIC1 was cleaved with biotinylated thrombin (Novagen, Madison, WI, USA). Cleaved GST and thrombin was removed by glutathione Sepharose 4B (Pharmacia, Uppsala, Sweden) and streptavidin-agarose, respectively. The resulting cleaved and purified NCC27/CLIC1 was used as an immunogen injected into rabbits. Three injections were performed every two weeks. Two weeks after the last immunization, sera were collected.

Cell culture and transfection

Human embryonic kidney (HEK293), HeLa, and C_2C_{12} cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. HEK293 cells were transiently transfected with the expression plasmid by a calcium phosphate precipitation method. Cells were incubated for 8 h with the transfection solution, washed with PBS, returned to culture with fresh media, and grown for a further 24 h. HeLa, and C_2C_{12} cells were transiently transfected by Lipofectamine™ (Life Technologies, Gaithersburg, MD, USA) according to the manufacturer's instructions.

Immunoblot analysis

Transfected HEK293 cells were lysed in 50 mM Tris (pH 8.0) and 0.5% NP-40 containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 2 mM aprotinin, and 2 mM leupeptin). Lysates were cleared by centrifugation at 15,000 g for 10 min. Protein contents of the resulting supernatants were determined by BCA™ kit (Pierce, Rockford, IL, USA). Proteins (20 g per lane) were separated by SDS-PAGE using 10% polyacrylamide gels, transferred to nitrocellulose membrane, and probed with the monoclonal antibody, SRT10 (0.1, 1, 10, 100, or 1000 ng/ml). Untransfected cell lysate used as a control

was probed with SRT10 at 1 g/ml. Sites of antibody binding were visualized by probing with alkaline phosphatase-conjugated goat anti-mouse IgG antibody (16 ng/ml, Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by colorimetric detection with nitroblue tetrazolium and bromochloroindolyl phosphate.

Immuoprecipitation

Preparation of transfected cell lysates was performed with the same methods as above. Immuoprecipitation were performed by adding the SRT10 (4 g) to cell lysates (150 g) and incubating for an hour at 4℃ with constant rotation. Protein A-Sepharose beads (Pharmacia, Uppsala, Sweden) were then added, and incubated in the same condition for one more hour. Immune complexes were then centrifuged for 2 min at 4,000 g, washed twice in lysis buffer and twice in washing buffer (lysis buffer with 0.25, 0.5, 0.75, 1 or 1.5 M NaCl), and resuspended in SDS gel-loading buffer (50mM Tris-HCl at pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol). Another monoclonal antibody to the SLP of *R. typhi* (IgG2a) was also used as a control for the precipitation and washed in washing buffer with 0.25 M NaCl. Samples were then analyzed by SDS-PAGE and immunoblotting. Blotted membrane was probed by the anti-NCC27/CLIC1 antibody described above. Alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (0.4 g/ml, Santa Cruz biotechnology, Santa Cruz, CA USA) was used to detect antibody-binding sites.

Immunocytochemistry

HeLa and C_2C_{12} cells grown on microscope cover glasses and transfected with the expression plasmid of SRT-NCC27/CLIC1 were fixed with 4% paraformaldehyde in PBS and permeabilized with 0.1% Triton X-100.

After washing in PBS, cells were incubated in SRT10 (3 g/ml), washed three times with PBS, incubated in FITC-conjugated goat anti-mouse IgG antibody (30 g/ml, ICN Biochemicals, Aurora, OH, USA), washed three times with PBS, and mounted with FlouroGuard™ (BioRad, Hercules, CA USA). Prepared cells were examined by confocal microscopy (LSM 510, Carl Zeiss, Jena, Germany).

RESULT AND DISCUSSION

Epitope mapping

By the analysis of a series of deletion constructs of *slpT* gene of *R. typhi*, we determined the epitope recognized by the monoclonal antibody SRT10 as the ten amino acid residues, TFIGAIATDT (SRTag).

Immunoblot analysis

To test whether the SRTag inserted in different sequence environment would affect its antigenicity, this epitope was fused in frame to the N-terminus of NCC27/CLIC1. This plasmid construct was transfected and the SRT-NCC27/CLIC1 was expressed in HEK293 cells. On measuring the protein content, 20 g of total cell lysate was subjected to immunoblotting. As shown in FIG 2, SRT10 recognized the SRT-NCC27/CLIC1 even at 1 ng/ml of antibody concentration. Detectable cross-reactivity with the HEK293 proteins was not observed. When the antibody-binding site was probed with the goat anti-mouse IgG antibody at 80 ng/ml, this secondary antibody cross-reacted with several cellular proteins. However, at this concentration, signals from the tagged proteins were recognized at 0.1 ng/ml of SRT10 (data not shown). When we expressed the deletion constructs of SLP in *E. coli* and examined their products with immunoblotting, no detectable

cross-reactivity with *E. coli* proteins was observed (data not shown). Thus this MAb can recognize the denatured tagged protein sensitively and specifically by immunoblotting.

Immunoprecipitation

To test whether SRT10 could precipitate tagged protein from mammalian cell lysate, transfected 293HEK cells were subjected to immunoprecipitation (FIG 3). Compared with the signal by the SRT-NCC27/CLIC1 from total cell lysate (FIG 3, lane 1), about 70% of tagged proteins were precipitated by 4 g of SRT10 in this condition. When the concentrations of NaCl in washing buffers were increased from 0.25 M to 1.5 M, the amount of precipitated SRT-NCC27/CLIC1 did not decrease suggesting a high affinity binding of the SRT10 to the SRT-tagged NCC27/CLIC1. When the cell lysate was precipitated with a control antibody, precipitation of the tagged protein was not observed (FIG 3, lane 2). Thus this antibody can precipitate the tagged protein from mammalian cell lysate efficiently.

Immunocytochemistry

To test whether SRT10 could detect intracellular tagged protein, transfected HeLa and C_2C_{12} cells were subjected to immunocytochemistry and examined by confocal microscopy (FIG 4). Most of the C_2C_{12} cells were stained prominently in the cytoplasm; however, a few cells were stained prominently in the nucleus. In HeLa cells, the antibody stained dominantly the nucleus, however, some cells were stained dominantly in the cytoplasm. It is likely that during the cell cycle, the localization of the NCC27/CLIC1 may be changed between the nucleus and cytoplasm. In adjacent, nontransfected cells, no detectable staining was observed (data not shown). Thus this monoclonal antibody can detect the tagged protein by

immunocytochemistry.

In this report, we determined the linear epitope recognized by the mouse monoclonal antibody (SRT10) to the rickettsial protein, SLP. By tagging this epitope to NCC27/CLIC1, we showed that SRT10 could detect this tagged protein by immunoblotting, immunoprecipitation, or immunocytochemistry. As this monoclonal antibody is both sensitive and specific, this tag and its monoclonal antibody should be generally applicable for cellular and molecular research.

REFERENCES

1. Evan, G.I., Lewis, G.K., Ramsay, G., and Bishop, J.M. 1985. Isolation of monoclonal antibodies specific for human c-*myc* proto-oncogene product. Mol. Cell. Biol. 12:3610-3616.

2. Hahn, M.J., Kim, K.K., Kim, I., and Chang, W.H. 1993. Cloning and sequence analysis of the gene encoding the crystalline surface layer protein of *Rickettsia typhi*. Gene 133:129-133.

3. Tulk, B.M., Edwards, J.C. 1998. NCC27, a homolog of intracellular Cl-channel p64, is expressed in brush border of renal proximal tubule. Am. J. Physiol. 274:F1140-9

4. Valenzuela, S.M., Martin, D.K., Por, S.B., Robbins, J.M., Warton, K., Bootcov, M.R., Schofield, P.R., Campbell, T.J., and Breit, S.N. 1997. Molecular cloning and expression of a chloride ion channel of cell nuclei. J. Biol. Chem. 272:12575-82.

5. Wilson, I.A., Niman, H.L., Houghten, R.A., Cherenson, A.R., Connolly, M.L., and Lerner, R.A. 1984. The structure of an antigenic determinant in a protein. Cell 37:767-778.

SEQUENCE LISTING

<110> HAHN, MYONG-JOON

<120> NEW EPITOPE TAG RECOGNIZED BY A MONOCLONAL ANTIBODY TO *RICKETTSIA TYPHI*

<130>

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What is claimed is :

1. An epitope recognized by a mouse monoclonal antibody (MAb), SRT10, to the crystalline surface layer protein (SLP) of *Rickettsia typhi* comprising SRTag consisting of ten amino acid sequence [SEQ ID NO : 1].

2. The epitope recognized by a mouse monoclonal antibody (MAb) according to claim 1, wherein an oligonucleotide sequence [SEQ ID NO : 2] corresponding to SRTag is inserted to an expression vector.

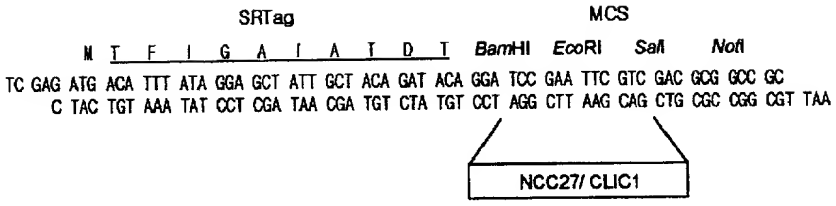
3. The epitope recognized by a mouse monoclonal antibody (MAb) according to claim 1, wherein the tagged protein is detected by MAb (SRT10) when the oligonucleotide sequence [SEQ ID NO : 2] corresponding to SRTag is fused to any protein gene desired to be expressed and detected in bacteria or mammalian cells.

4. Monoclonal or polyclonal antibody to the SRTag as an epitope for any tagged protein.

5. The application of epitope of claim 1 for the research of cellular and molecular biology requiring the expression and detection of fusion proteins.

ABSTRACT

The epitope recognized by a mouse monoclonal antibody (MAb) to the crystalline surface layer protein (SLP) of *Rickettsia typhi*, SRT10, is mapped to ten amino acid residues (SRTag, TFIGAIATDT). The oligonucleotide sequence covering the epitope recognized by SRT10 is inserted to a mammalian expression vector together with multiple cloning sites. When the SRTag is fused in frame to the coding region of protein gene and expressed in bacteria or mammalian cells, the MAb SRT10 can detect the tagged protein by immunoblotting, immunocytochemistry, and immunoprecipitation.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MCS
M T F I G A I A T D T
BamHI EcoRI SalI NotI
TC GAG ATG ACA TTT ATA GGA GCT ATT GCT ACA GAT ACA GGA TCC GAA TTC GTC GAC GCG GCC GC
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NCC27/ CLIC1

FIG 1

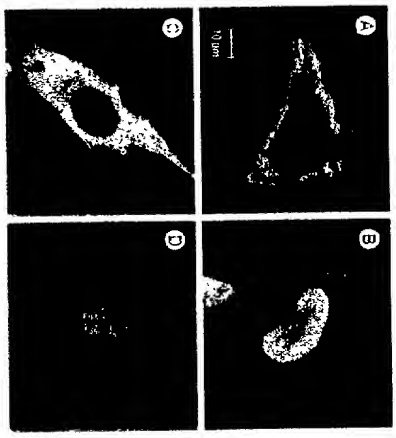

FIG 2

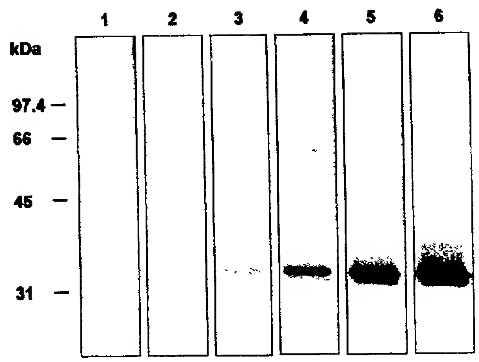

FIG 3

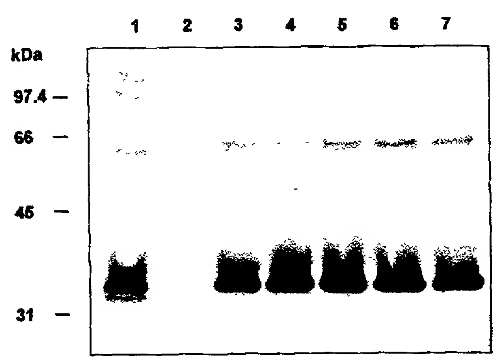

FIG 4

APPLICATION FOR UNITED STATES PATENT DECLARATION AND POWER OF ATTORNEY

Docket No 105997

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: ____

New epitope tag recognized by a monoclonal antibody to Rickettsia typhi

described and claimed in the specification:

Check one

*a. ☒ attached hereto.

b. ☐ filed on _____ as Application No. _____ and amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) and/or United States provisional application(s) filed by me or my legal representatives or assigns within one year prior to this application are hereby claimed:

The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to this application, or (b) before the filing date of the above-named foreign priority application(s) and/or United States provisional application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

James A. Oliff, Reg. No. 27,075; William P. Berridge, Reg. No. 30,024;
Kirk M. Hudson, Reg. No. 27,562; Thomas J. Pardini, Reg. No. 30,411;
Edward P. Walker, Reg. No. 31,450; Robert A. Miller, Reg. No. 32,771;
Mario A. Costantino, Reg. No. 33,565; Caroline D. Dennison, Reg. No. 34,494; and
Stephen J. Roe, Registration No. 34,463.

ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE, PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1 *Typewritten Full Name of First or Sole Inventor*: Myong-Joon (Given Name) | (Middle Initial) | HAHN (Family Name)

2 **Inventor's Signature: Myong-Joon Hahn

3 **Date of Signature: April 3, 2000 (Month / Day / Year)

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*If Box (a.) is checked, this form may be executed only when attached to the specification (including claims).

**Note to Inventor: Please sign name exactly as it appears above and insert actual date of signing.

IF THERE IS MORE THAN ONE INVENTOR USE PAGE 2 AND PLACE AN "X" HERE ☐

10/96

SEQUENCE LISTING

<110> HAHN, Myong-Joon

<120> NEW EPITOPE TAG RECOGNIZED BY A MONOCLONAL ANTIBODY TO RICKETTSIA TYPHI

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